

THE SLIME OF THE HOUSEHOLD BATH-SPONGE.

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Rhizobium limosospongiae, n.sp.

The sliming of household-sponges is a matter of common observation, and it appears to be the general idea that it is caused, in some way, by the soap used with the sponge. The explanation thus given is probably traceable to the difficulty experienced by the housekeeper in finding a more suitable explanation. Doubtless the formation of a lime-soap scum on water in basins and baths, induced the idea that a deposition of a similar substance occurs in the pores of the sponge. Probably the best means for removing the slime, without injuring the substance of the sponge too much, is to immerse it in hot, soft water, as this causes the slime to swell. The sponge is then squeezed in a cloth or towel, when the swollen slime oozes through the cloth. A repetition of this procedure will probably remove all the slime.

If one prepares and examines a stained film of the slime, one sees it is crowded with long, more or less bent, rod-like forms; and that the rods are swollen irregularly, very often with partially detached ends, characteristic of the *Rhizobium*-type of micro-organism.

When the slime was smeared upon plates of saccharose-bean-agar, the preparation of which has already been described,* a number of colonies of slime-forming bacteria developed in the course of a few days. The great majority of them were of the *Rhizobium*-type, and the slimes varied in consistency. The

* These Proceedings, 1906, p.267.

original colonies were by no means pure. Some contained *Radio-bacter*, which grows best in combination with other bacteria. Others contained *Sarcinae* and short rods. A second smearing, followed by the usual dilution-method of plating bacteria, enabled pure cultures to be obtained. There were several kinds of *Rhizobia*, some stouter some thinner, but all produced what appeared to be a similar kind of slime. One of the organisms was taken for further work. A bacterium forming a tough slime was taken as a second microbe because it was probable that this was the cause of the slime being so adhesive to the sponge. The latter micro-organism appeared to lie midway between *Rhizobium* and *Bac. alatus* Greig-Smith.

The two bacteria were smeared upon a synthetic agar-medium containing phosphate and asparagin in combination with various sugars, etc., in order to see which sugar to use in preparing a quantity of the slime. The tough slime-bacterium refused to grow, while the other (*Bac.ii.*) produced luxuriant slimes from saccharose, levulose, and dextrose, and no slime from lactose, glycerin, dextrin, maltose or mannit. Another test was made, using bean-extract as a basis, with the same result as far as the loose slime-bacterium was concerned. The tough slime-bacterium (*Bac. i.*) produced a luxuriant slime in the presence of saccharose, levulose, glycerin, mannit and maltose, but failed to respond to lactose and dextrin.

These slimes were used to prepare films for microscopical observation. Both bacteria were Gram-negative and varied very much in size and shape, not only with regard to different sugars, but even with each sugar; and, upon the same film, great variations were seen.

With *Bacillus i.*, a smaller or larger oval shape predominated. From glycerin, the cells were comparatively small and stained deeply; from the other media, they stained diffusely. From maltose and dextrose, types similar to *Bac. alatus** with winged capsules were common. Levulose and mannit favoured the

* These Proceedings, 1905, p.570.

formation of oval cells which remained attached, in groups of three and four, as short chains, the individuals of which varied in size; and sometimes the terminal pair were at right angles to the others, thus producing a T- or Y-shape.

Bacillus ii. rarely exhibited the *alatus*-form. The typical cell was oval and stained terminally. Mixed with these were bent rods staining irregularly and evidently containing two, three, or four oval cells within a rod-shaped capsule. The exclamation-mark (!) form was generally seen, and in some films Y-forms were noted. It was clearly of the *Rhizobium*-type, while Bac. i. was evidently allied.

The respective slimes were obtained in quantity by growing Bac. i. on saccharose-bean-agar, and Bac. ii. on levulose-asparagin-agar. From these slimes, gums were prepared according to the method used in the preparation of other gums.* The slime squeezed from the sponge was precipitated with alcohol, treated with water, and made to yield its gum in the same fashion. All the gums were tested with various reagents with the following results.

	Sponge gum.	Bac. i. gum.	Bac. ii. gum.
Alcohol.....	+	+	+
Basic lead acetate.....	+	+	+
Ammoniacal lead acetate	+	+	+
Neutral lead acetate.....	×	×	×
Barium hydrate.....	×	×	×
Ferric chloride.....	+	+	+
Copper sulphate.....	0	0	0
CuSO ₄ (dil.) followed by KOH.....	+	+	+
Phosphotungstic acid.....	×	×	+
Fehling's solution.....	+	+	+
Milk of lime.....	0	0	0
Silver nitrate.....	0	0	?
Iodine	0	0	0
Tannic acid.....	?	?	?
Sulphuric acid.....	0	0	0
Sodium hydrate.....	0	0	0

+ = coagulation; × = a precipitate; ? = opalescence; 0 = no reaction.

* These Proceedings, 1906, p. 268.

These gums are similar to one another, and are probably identical. They are somewhat similar to the gum obtained from *Rhizobium leguminosarum*, excepting in one or two reactions, as, for example, Fehling's solution, with which the *Rhizobium*-gum does not coagulate. Otherwise *Rhizobium*-gum forms a jelly, while these sponge-gums form mucilages.

The bacterial gums were completely hydrolysed by boiling with 5% sulphuric acid, during which process furfural was evolved. Before and after hydrolysis, the gum of Bac. ii., which had been grown from levulose, was dextrorotatory. The solutions of the sugars, obtained after neutralising the sulphuric acid with barium carbonate and evaporating the filtrate to small bulk, were treated with phenylhydrazine-acetic acid mixture, and the impure osazones filtered off.

These were purified by the method already described,* and found to consist of galactosazone. No other osazone could be detected, although crystalline osazone-like bodies with indefinite melting points (140° to 155°) were obtained. I have previously suggested that these are probably derived from the furfuroid or similar bodies. Upon oxidation with nitric acid the gums yielded a mixture of oxalic and mucic acids. Thus the gums were galactans.

Bac. No. i. formed a dry, rough, scaly, glistening growth on nutrient agar. In bouillon it grew scantily, chiefly as a surface ring, and the fluid remained clear; nitrates were reduced to nitrites and indol was formed. Litmus-milk was bleached, and the casein slowly peptonised, the reaction becoming alkaline, especially at the surface, where the dried casein appeared as a broad, deep blue ring. In glucose-gelatin, the growth was translucent and rough, the medium becoming slowly liquefied; the colonies were translucent and moruloid. In nutrient gelatin, the growth was similar, but there was no liquefaction. On potato there formed a transparent, flat, glistening growth which

* These Proceedings, 1902, p.394, and 1903, p.546.

became brownish. In Lemco-broth, with various sugars, the bacteria formed surface-rings, but there was no production of acid.

Bacillus No. ii. (*Rhizobium limosopongiae*, n.sp.) formed, on nutrient agar, a very scanty, slowly growing collection of isolated colonies, which slowly fused as a translucent, glistening, raised growth. The cells were entirely of the bacteroid type, as found in the tubercles of certain Leguminosæ. The growths in bouillon were scanty, nitrates were reduced to nitrites, and indol was formed. Litmus-milk became alkaline. The bacterium would not grow on gelatin, either glucose or nutrient. On potato, the growth was dull white, flat, and scanty. In Lemco-bouillon containing saccharose, dextrose, levulose, lactose, mannit or glycerin, surface-rings were formed, but there was no production of acid. No motility was ever observed.

While the bacteria are capable of producing slime upon the surface of certain nutritive agar media, they are apparently incapable of doing so when immersed in fluid. This was shown in an experiment in which the bacteria were grown, for three weeks, in a faintly acid solution containing asparagin(0.06 %), sodium phosphate(0.2 %), and levulose or dextrose(2 %). This nutrient fluid favoured the formation of branched forms of *Rhizobium leguminosarum*, but did not do so with these two bacteria. In the fluids, which were a centimetre deep, no slime was produced; and a similar absence of slime was noted in the cultures in Lemco-bouillon with various carbonaceous nutrients.

The condition, as regards the supply of oxygen, will be very much the same upon the surface of a damp sponge, as upon the surface of agar, while the nutrients are quite different. The question, therefore, arises, from what substance is the slime elaborated in the sponge? The solids of the water will supply the necessary saline matter, and it is conceivable that the traces of nitrogenous matter derived from the skin may be the source of the carbon. It may be the soap, but one does not expect that a sodium fatty acid salt would do this. In experiments with fluid and solid (agar) media containing asparagin and soap, no



slime was obtained. Soap* is, therefore, out of the question as a source of slime.

The most feasible explanation is, that the substance of the sponge serves as a source both of carbon and of nitrogen. It consists chiefly of spongin, a proteid which contains 16 % of nitrogen. This is the more probable as the texture of a sponge, which has been attacked, is appreciably softer after the slime has been removed.

The activity of the bacteria in producing slime from sponge-substance, was tested by using four sponges, one of which was infected with an emulsion of Bac. i., another with Bac. ii., and a third with a mixture of both, while a fourth was reserved as a control. All were kept moist in covered beakers at 27°. In 17 days the sponge infected with Bac. ii. was a sodden, slimy mass, and had shrunk considerably. The control was unaffected, while the others were slightly slimy. The behaviour of the sponge with the mixture of bacteria, was explained by its having had a slight infection, the remains of what had not been soaked up by the first sponge. Subsequently it became much more slimy. Although the experimental sponge had been reduced in size and elasticity, there was no apparent change in the microscopical appearance of the network; the only difference noted between the infected and the control sponges was that, in the former, masses of bacteria-containing slime filled up the spaces of the sponge. The slime from the sponge contained great numbers of rod-forms, of various dimensions, and staining

* If a slimy sponge is rubbed with soap, squeezed, and rubbed up again and again, one can obtain a condition in which the slime appears to have been removed, and the sponge behaves, to soapy water, as if no slime were in it. The slime, however, has not been removed; it has simply been coagulated, and the sponge will regain its slimy consistence on removal of the soap with changes of fresh, soft water. A sponge which is slightly slimy in hard water, becomes very slimy when soft water is used. These facts show that the slime is coagulated by soap, and by various salts. The same thing was noted when fragments of slime were inserted in nutrient bouillon; they did not swell and diffuse, but appeared to become coagulated, and remained in the medium as lumps of slime.

irregularly, like the original bacteria used for infecting. Others appeared as short, oval bacteria contained in a long, sometimes thick, sometimes thin, cylindrical capsule, the whole resembling a *Streptococcus*. Plate-cultivation showed a mixed culture of bacteria, some producing no slime, and others forming raised, compact colonies of the irregularly staining rods imbedded in a tough slime. These were similar to the infecting bacteria, although the slime was tougher.

It was evident that the growth upon the sponge had altered the character of the bacillus, to the extent of causing it to produce a more viscous slime. Further examination showed that the growth-characters had also altered. The growths upon media were much more vigorous. It now grew upon glucose and nutrient gelatin as a dull, rough layer, slowly liquefying the medium. In litmus-Lemco-gelatin with various sugars, acid and gas were produced from dextrose, saccharose, and mannit, while lactose was unaffected. A dry, yellowish layer formed on potato.

Summary.—The sliminess of the household-sponge is caused by slime-forming bacteria, one of which, *Rhizobium limosospongiae* n.sp., has been shown to be an active agent in producing the phenomenon.